CLAIMS

We claim:

1. A chimeric protein comprising:

(a) a Kunitz-type domain 1 of TFPI-2, and

(b) a Kunitz-type domain 2 of TFPI.

2. The chimeric protein of claim 1, wherein said chimeric protein is represented by the generic structure:

$A-(X_1)_a-B-(X_2)_b-C$

wherein A and C are independently optional flanking peptides, the flanking peptides containing 0-100 amino acids;

wherein B is an optional spacer peptide, the spacer peptide containing 0-25 amino acids;

wherein each X_1 is $-D-K_1-E-$

where D,E are independently polypeptides of 0-25 amino acids,

where K_1 comprises TFPI Kunitz-type domain 1 or a mutein thereof, or TFPI-2 Kunitz-type domain 1 or a mutein thereof;

wherein each X_2 is $-F-K_2-G-$

where F,G are independently polypeptides of 0-25 amino acids,

where K_2 comprises TFPI Kunitz-type domain 2 or a mutein thereof, or TFPI-2 Kunitz-type domain 2 or a mutein thereof;

wherein a,b are integers from 0-6;

wherein A,B,C,D,E,F,G may comprise portions of native TFPI or TFPI-2 sequences or non-native sequence; and

and the molecule is not native TFPI or TFPI-2.

3. The chimeric protein of claim 2, wherein A or C comprises Kunitz-type domain 3 of TFPI.

4. The chimeric protein of claim 2, wherein A or C comprises Kunitz-type domain 3 of TFPI-2.

5. The chimeric protein of claim 2, wherein at least one of said flanking peptides comprises an amino acid sequence capable of binding one or more cell surface components.

6. The chimeric protein of claim 5, wherein said amino acid sequence capable of binding one or more cell surface components is an amino acid sequence capable of binding a glycosaminoglycan.

7. The chimeric protein of claim 6, wherein said amino acid sequence capable of binding a glycosaminoglycan is an amino acid sequence capable of binding heparin.

8. The chimeric protein of claim 7, wherein said amino acid sequence capable of binding heparin is a heparin-binding domain from a protein, said protein selected from the group consisting of:

(a) protease nexin-1;
(b) protease nexin-2;
(c) antithrombin III;
(d) heparin cofactor II;
(e) protein C inhibitor;
(f) platelet factor 4;
(g) bovine pancreatic trypsin inhibitor; and
(h) ghilanten-related inhibitors.

9. The chimeric protein of claim 7, wherein said amino acid sequence capable of binding heparin is a heparin-binding domain selected from the group consisting of:

(a) SEQ ID NO: 10;
(b) SEQ ID NO: 11;

(c) SEQ ID NO: 12;

(d) SEQ ID NO: 13;

(e) SEQ ID NO: 14;

(f) SEQ ID NO: 15;

(g) SEQ ID NO: 16;

(h) SEQ ID NO: 17; and

(i) SEQ ID NO: 18.

10. The chimeric protein of claim 5, wherein said flanking peptide comprises the C-terminal tail of TFPI [SEQ ID NO: 7].

11. The chimeric protein of claim 5, wherein said flanking peptide comprises the C-terminal tail of TFPI-2 [SEQ ID NO: 8].

12. The chimeric protein of claim 2, wherein each K_1 is Kunitz-type domain 1 of TFPI-2, each K_2 is Kunitz-type domain 2 of TFPI, and a and b are integers greater than 1.

13. The chimeric protein of claim 2, wherein each K1 is a mutein of Kunitz-type domain 1 of TFPI-2, each K2 is a mutein of Kunitz-type domain 2 of TFPI, and a and b are integers greater than 1.

14. The chimeric protein of claim 1, wherein the primary amino acid sequence of the chimeric protein is SEQ ID NO: 19.

15. The chimeric protein of claim 1, wherein the chimeric protein comprises first and second amino acid sequences, said first amino acid sequence comprising SEQ ID 19 and said second amino acid sequence selected from the group consisting of:

(a) SEQ ID NO: 7;

(b) SEQ ID NO: 8;

(c) SEQ ID NO: 10;

(d) SEQ ID NO: 11;

(e) SEQ ID NO: 12;

(f) SEQ ID NO: 13;

(g) SEQ ID NO: 14;

(h) SEQ ID NO: 15;

(i) SEQ ID NO: 16;

(j) SEQ ID NO: 17; and

(k) SEQ ID NO: 18.

16. The chimeric protein of claim 1, wherein said chimeric protein is represented by the generic structure:

$A - [X_1 - B - X_2]_c - C$

wherein A and C are optional flanking peptides, the flanking peptides independently containing 1-100 amino acids;

wherein B is an optional spacer peptide, the spacer peptide containing 1-25 amino acids;

wherein each X_1 is -D-K_1-E-

where D,E are independently peptides of 1-25 amino acids,

where K_1 is Kunitz-type domain 1 from TFPI or TFPI-2 or a mutein of the aforementioned Kunitz-type domain;

wherein each X_2 is -F-K_2-G-

where F,G are independently peptides of 1-25 amino acids,

where K_2 is Kunitz-type domain 2 from TFPI or TFPI-2 or a mutein of the aforementioned Kunitz-type domain;

wherein c is an integer from 1-10.

17. The chimeric protein of claim 16, wherein A or C comprises Kunitz-type domain 3 of TFPI

[SEQ ID NO: 7].

18. The chimeric protein of claim 16, wherein A or C comprises Kunitz-type domain 3 of TFPI-2 [SEQ ID NO: 8].

19. The chimeric protein of claim 16, wherein at least one of said flanking peptides comprises an amino acid sequence capable of binding one or more cell surface components.

20. The chimeric protein of claim 19, wherein said amino acid sequence capable of binding one or more cell surface components is an amino acid sequence capable of binding glycosaminoglycan.

21. The chimeric protein of claim 20, wherein said amino acid sequence capable of binding glycosaminoglycan is an amino acid sequence capable of binding heparin.

22. The chimeric protein of claim 21, wherein said amino acid sequence capable of binding heparin is a heparin-binding domain from a protein, said protein selected from the group consisting of:

(a) protease nexin-1;
(b) protease nexin-2;
(c) antithrombin III;
(d) heparin cofactor II;
(e) protein C inhibitor;
(f) platelet factor 4;
(g) bovine pancreatic trypsin inhibitor; and
(h) ghilanten-related inhibitors.

23. The chimeric protein of claim 21, wherein said amino acid sequence capable of binding heparin is a heparin-binding domain selected from the group consisting of:

(a) SEQ ID NO: 10;

(b) SEQ ID NO: 11;

(c) SEQ ID NO: 12;

(d) SEQ ID NO: 13;

(e) SEQ ID NO: 14;

(f) SEQ ID NO: 15;

(g) SEQ ID NO: 16;

(h) SEQ ID NO: 17; and

(i) SEQ ID NO: 18.

24. The chimeric protein of claim 19, wherein said flanking peptide comprises the C-terminal tail of TFPI [SEQ ID NO: 7].

25. The chimeric protein of claim 19, wherein said flanking peptide comprises the C-terminal tail of TFPI-2 [SEQ ID NO: 8].

26. The chimeric protein of claim 1 wherein said protein is produced in a yeast cell and contains no carbohydrate which is immunogenic in mammals.

27. The chimeric protein of claim 26 wherein said protein contains no α-1,6-polymannose terminal carbohydrate.

28. A TFPI mutein, said TFPI mutein having a one or more substitutions, said one or more substitutions occurring exclusively in the P1-reactive site in one or more Kunitz-type domains.

29. The TFPI mutein of claim 28 wherein said one or more substitutions is one substitution.

30. The TFPI mutein of claim 28 wherein said mutein has the primary amino acid sequence of

SEQ ID NO: 9.

31. A TFPI-2 mutein, said TFPI mutein having a one or more substitutions, said one or more substitutions occurring exclusively in the P1-reactive site in one or more Kunitz-type domains.

32. The TFPI-2 mutein of claim 31 wherein said one or more substitutions is one substitution.

33. The TFPI-2 mutein of claim 27 wherein said one substitution is a Glu to Arg substitution at the P1 reactive site of Kunitz-type domain 2 [SEQ ID NO: 4].

34. A nucleic acid sequence comprising nucleic acids encoding the chimeric protein of claim 1.

35. A nucleic acid sequence comprising nucleic acids encoding claim 28.

36. A nucleic acid sequence comprising nucleic acids encoding claim 31.

37. An expression vector capable of producing the chimeric protein of claim 1, said expression vector comprising the nucleic acid sequence of claim 34 and expression control sequences compatible for expression of said chimeric protein.

38. An expression vector capable of producing the TFPI mutein of claim 28, said expression vector comprising the nucleic acid sequence of claim 35 and expression control sequences compatible for expression of the TFPI mutein of claim 28.

39. An expression vector capable of producing the TFPI-2 mutein of claim 31, said expression vector comprising the nucleic acid sequence of claim 36 and expression control sequences compatible for expression of the TFPI mutein of claim 31.

40. A transformed host cell capable of producing a chimeric protein, said transformed host cell comprising the expression vector of claim 37 wherein said expression vector further comprises expression control sequences operably linked to an expression control sequence operable in said host cell.

41. A transformed host cell capable of producing the TFPI mutein of claim 28, said transformed host cell comprising the expression vector of claim 38 wherein said expression vector further comprises expression control sequences operably linked to an expression control sequence operable in said host cell.

42. A transformed host cell capable of producing the TFPI mutein of claim 31, said transformed host cell comprising the expression vector of claim 39 wherein said expression vector further comprises expression control sequences operably linked to an expression control sequence operable in said host cell.

43. A method of producing the chimeric protein of claim 1, said method comprising:

(a) providing a transformed host cell comprising an expression vector comprising a polynucleotide sequence encoding the chimeric protein and further comprises expression control sequences compatible for expression of the polynucleotide sequence encoding the chimeric protein of claim 1;

(b) incubating said transformed host cell under conditions that allow expression of said polynucleotide sequence encoding the chimeric protein of claim 1.

44. The method of claim 43 wherein said host cell is a yeast cell.

45. The method of claim 44 wherein said yeast cell is selected from the group consisting of

Candida albicans; *Candida maltosa*; *Hansenula polymorpha*; *Kluyveromyces fragilis*; *Kluyveromyces lactis*; *Pichia guillerimondii*; *Pichia pastoris*; *Saccharomyces cerevisiae*; *Schizosaccharomyces pombe*; and *Yarrowia lipolytica*.

46. The method of claim 45 wherein said yeast cell is *Saccharomyces cerevisiae*.

47. The method of claim 46 wherein said protein is retained within the yeast cell.

48. The method of claim 47 wherein said polynucleotide sequence encoding said protein is immediately preceded in frame by a polynucleotide sequence encoding ubiquitin.

49. The method of claim 43 wherein said protein is secreted from the yeast cell.

50. The method of claim 49 wherein said polynucleotide sequence encoding said protein is immediately preceded in frame by a polynucleotide sequence encoding yeast α factor.

51. The method of claim 43 wherein said yeast cell is prevented from producing α-1,6-polymannose terminal carbohydrate.

52. The method of claim 51 wherein said yeast cell carries *och1*, *mnn1* and *alg3* mutations.

53. A method of producing the TFPI mutein of claim 28, said method comprising:

(a) providing a transformed host cell comprising an expression vector comprising a polynucleotide sequence encoding the TFPI mutein of claim 28 and further comprises expression control sequences compatible for expression of said polynucleotide sequence encoding the TFPI mutein of claim 28; and

(b) incubating said transformed host cell under conditions that allow expression of said polynucleotide sequence encoding the TFPI mutein of claim 28.

54. The method of claim 53 wherein said host cell is a yeast cell.

55. The method of claim 54 wherein said yeast cell is selected from the group consisting of *Candida albicans*; *Candida maltosa*; *Hansenula polymorpha*; *Kluyveromyces fragilis*; *Kluyveromyces lactis*; *Pichia guillerimondii*; *Pichia pastoris*; *Saccharomyces cerevisiae*; *Schizosaccharomyces pombe*; and *Yarrowia lipolytica*.

56. The method of claim 55 wherein said yeast cell is *Saccharomyces cerevisiae*.

57. The method of claim 56 wherein said protein is retained within the yeast cell.

58. The method of claim 57 wherein said polynucleotide sequence encoding the TFPI mutein of claim 28 is immediately preceded in frame by a polynucleotide sequence encoding ubiquitin.

59. The method of claim 53 wherein said protein is secreted from the yeast cell.

60. The method of claim 59 wherein said polynucleotide sequence encoding the TFPI mutein of claim 28 is immediately preceded in frame by a polynucleotide sequence encoding yeast α factor.

61. The method of claim 53 wherein said yeast cell is prevented from producing α-1,6-polymannose terminal carbohydrate.

62. The method of claim 61 wherein said yeast cell carries *och1*, *mnn1* and *alg3* mutations.

63. A method of producing the TFPI-2 mutein of claim 31, said method comprising:

(a) providing a transformed host cell comprising an expression vector comprising a polynucleotide sequence encoding the TFPI-2 mutein of claim 31, said expression vector further comprising expression control sequences compatible for expression of said polynucleotide sequence encoding the TFPI-2 mutein of claim 31; and

(b) incubating said transformed host cell under conditions that allow expression of said polynucleotide sequence encoding encoding the TFPI-2 mutein of claim 31.

64. The method of claim 63 wherein said host cell is a yeast cell.

65. The method of claim 64 wherein said yeast cell is selected from the group consisting of *Candida albicans*; *Candida maltosa*; *Hansenula polymorpha*; *Kluyveromyces fragilis*; *Kluyveromyces lactis*; *Pichia guillerimondii*; *Pichia pastoris*; *Saccharomyces cerevisiae*; *Schizosaccharomyces pombe*; and *Yarrowia lipolytica*.

66. The method of claim 65 wherein said yeast cell is *Saccharomyces cerevisiae*.

67. The method of claim 66 wherein said protein is retained within said yeast cell.

68. The method of claim 67 wherein said polynucleotide sequence encoding the TFPI-2 mutein of claim 31 is immediately preceded in frame by a polynucleotide sequence encoding ubiquitin.

69. The method of claim 66 wherein said protein is secreted from the yeast cell.

70. The method of claim 69 wherein said polynucleotide sequence encoding said protein is immediately preceded in frame by a polynucleotide sequence encoding yeast α factor.

71. The method of claim 66 wherein said yeast cell is prevented from producing α-1,6-polymannose terminal carbohydrate.

72. The method of claim 71 wherein said yeast cell carries *och1*, *mnn1* and *alg3* mutations.

73. A pharmaceutical composition comprising the chimeric protein of claim 1 and a pharmaceutically acceptable carrier.

74. A pharmaceutical composition comprising the TFPI mutein of claim 28 and a pharmaceutically acceptable carrier.

75. A pharmaceutical composition comprising the TFPI-2 mutein of claim 31 and a pharmaceutically acceptable carrier.

76. A method of treating septic shock comprising administering the pharmaceutical composition of claim 68 to a mammal in an effective amount.

77. A method of treating septic shock comprising administering the pharmaceutical composition of claim 69 to a mammal in an effective amount.

78. A method of treating septic shock comprising administering the pharmaceutical composition of claim 70 to a mammal in an effective amount.

79. A method of treating thrombosis disorders comprising administering the pharmaceutical composition of claim 68 to a mammal in an effective amount.

80. A method of treating thrombosis disorders comprising administering the pharmaceutical composition of claim 69 to a mammal in an effective amount.

81. A method of treating thrombosis disorders comprising administering the pharmaceutical composition of claim 70 to a mammal in an effective amount.

82. A monoclonal antibody, said monoclonal antibody capable of selectively binding to the chimeric protein of claim 1 .

83. The monoclonal antibody of claim 82 wherein said antibody has an affinity constant of at least 10^7 /M.

84. A monoclonal antibody, said monoclonal antibody capable of selectively binding to the TFPI mutein of claim 28.

85. The monoclonal antibody of claim 84 wherein said antibody has an affinity constant of at least 10^7 /M.

86. A monoclonal antibody, said monoclonal antibody capable of selectively binding to the TFPI-2 mutein of claim 31.

87. The monoclonal antibody of claim 86 wherein said antibody has an affinity constant of at least 10^7 /M.

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